Bohn, Brent

From: Cowden, John
Sent: Monday, March 31, 2014 2:53 PM
To: Davis, Allen; Gift, Jeff; Luben, Tom; Kirrane, Ellen
Cc: Lee, Janice; Sams, Reeder
Subject: FW: Arsenic: Proposed Approach for Meta-Analyses
Attachments: Arsenic_Meta-Analyses Approach_032814.pdf

Categories: Record Saved - Private

Hi Ellen, Allen, Jeff, and Tom,

Happy Monday! I hope that things are going well for you today.

ICF has pulled together a draft approach for hazard ID meta-analyses. If you have time, take a quick look and see what you think. Mostly, I just wanted the DREAMers to have a copy.

Let me know if you have any questions. Have a great afternoon!

John

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(919) 541-3667

From: Turley, Audrey [mailto:Audrey.Turley@icfi.com]
Sent: Friday, March 28, 2014 6:23 PM
To: Cowden, John; Lee, Janice; Sams, Reeder
Cc: Eftim, Sorina; Mendez Jr, William; Burch, Dave
Subject: Arsenic: Proposed Approach for Meta-Analyses

John, Janice, and Reeder,

The attached memo describes our proposed approach for meta-analyses for hazard identification. We look forward to discussing it with you on either Thursday or Friday of next week.

Are you available at any of these times?
Thursday, April 3
- 11-12
- 2:30-3:30

Friday, April 4
- 11-12
- 2-3
- 3-4

Thank you,
Audrey

AUDREY TURLEY |

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Bohn, Brent

From:	Cowden, John
Sent:	Friday, February 07, 2014 1:32 PM
To:	Turley, Audrey
Subject:	FW: Arsenic: Resolutions and Action Items for Evidence Tables and SQ Review
Attachments:	As_EPA Comments on Evidence Tables and SQ - Resolutions and Action Items_013114 _tjl.docx
Categories:	Record Saved - Private

Hi Audrey,

Happy Friday! I hope that things are going well for you today.

Here is the INITIAL DRAFT DRAFT DRAFT version of Tom and Ellen's comments. They will be updated in the near future.

Have a great afternoon!

John

From: Luben, Tom
Sent: Monday, February 03, 2014 9:19 AM
To: Kirrane, Ellen
Cc: Cowden, John; Sams, Reeder
Subject: FW: Arsenic: Resolutions and Action Items for Evidence Tables and SQ Review

Ellen,

I went through the document that Audrey sent and made some comments in green.

Do you have time to meet this afternoon to go through these?

Thanks!

Tom

From: Turley, Audrey [mailto:Audrey.Turley@icfi.com]
Sent: Sunday, February 02, 2014 4:30 PM
To: Cowden, John; Lee, Janice; Sams, Reeder; Kirrane, Ellen; Luben, Tom
Cc: Burch, Dave; Marin, Kristen; Eftim, Sorina; Blain, Robyn
Subject: Arsenic: Resolutions and Action Items for Evidence Tables and SQ Review

Hello,
The attached notes summarize the action items and resolutions for the evidence tables and study quality review from our meeting on Thursday, January 30.
Please confirm that you received the file.
Thank you,
Audrey

AUDREY TURLEY |
ICF INTERNATIONAL |

Bohn, Brent

From:	Cowden, John
Sent:	Wednesday, March 06, 2013 3:45 PM
To:	Sams, Reeder
Subject:	FW: draft ADP for iAs
Categories:	Record Saved - Private

Hey Reeder,

Happy Wednesday! I hope that things are going well for you today.

Here is the draft analysis plan. The analysis plan is the detailed path for evaluating arsenic data. It fleshes out the overall path described in the conceptual model (which I will send you at some point.... ☺)

Let me know if you have any questions. Have a great evening!

John

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From: Lee, Janice
Sent: Friday, February 08, 2013 1:29 PM
To: Cowden, John; Sams, Reeder
Subject: draft ADP for iAs

Happy Friday fellow summiters!! I am so excited b/c I just got a big dry erase board put up in my office.... it is so nice! (what has my life become?)
Anyway I have been working on my section of the ADP, and I'm sending it along now b/c I don't want to look at it anymore. and I could use some feedback!
thanks! and have a good weekend!

Sunday is chinese new year so happy year of the snake to you!

Janice

iAs Problem Formulation Sta...

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Bohn, Brent

From: Lee, Janice
Sent: Friday, April 19, 2013 12:54 PM
To: Sams, Reeder; Cowden, John
Subject: RE: ADP

Categories: Record Saved - Private

Hi Reeder,

The figures are jpg files. I have the jpg files and can revise the titles if you let me know what they should be. I can re-insert them into the latest draft or just send you the revised jpg files. Let me know.

Janice

From: Sams, Reeder
Sent: Friday, April 19, 2013 12:45 PM
To: Cowden, John; Lee, Janice
Subject: ADP

John and Janice,

Attached is the current draft of the ADP. I have made it through Section 4 and am starting to go through Section 5. In Section 5.5.1.2 it has a note to check with Ryan, does this text need revised? Also, I tried to revise the figure titles in Section 4, but it would not allow me to go to the original file. My plan is to send out hopefully today or before Monday.

Best Regards,
Reeder

Reeder L. Sams II, Ph.D.
Deputy Director (Acting)
Research Triangle Park Division
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency, B243-01
RTP, NC 27711

Phone: 919-541-0661
Fax: 919-541-0245

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Bohn, Brent

From:	Cowden, John
Sent:	Monday, February 24, 2014 2:50 PM
To:	Turley, Audrey
Cc:	Burch, Dave; Sams, Reeder; Lee, Janice
Subject:	RE: Arsenic - Revised lit search and systematic review documentation
Categories:	Record Saved - Private

Hi Audrey,

Happy Monday! I hope that things are going well for you today.

We did receive the materials from Dave on Friday. I have incorporated the revisions into the draft ADP, although I still prefer the RoB criteria in table format (i.e. epi section) over the text format (animal section). ☺

Let me know if you have any questions. Have a great afternoon!

John

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From: Turley, Audrey [mailto:Audrey.Turley@icfi.com]
Sent: Monday, February 24, 2014 12:39 PM
To: Cowden, John; Sams, Reeder; Lee, Janice
Cc: Burch, Dave
Subject: FW: Arsenic - Revised lit search and systematic review documentation

John, Janice, and Reeder,
Can you confirm that you received the attached file on Friday? And that it's not sitting in junk...
Thanks,
Audrey

From: Burch, Dave
Sent: Friday, February 21, 2014 12:31 PM
To: Cowden, John (Cowden.John@epa.gov)
Cc: Turley, Audrey; Lee, Janice (Lee.JaniceS@epa.gov); Reeder Sams (sams.reeder@epa.gov)
Subject: Arsenic - Revised lit search and systematic review documentation

Hi John,

Attached is the updated documentation of the literature review/evaluation process. This was previously referred to as the "lit search strategy" document, but I've changed the name to better reflect what it now contains.

There are a few places that require updating. One is the lit search flow diagram; this is still in progress, and we'll get that to you later today. There are also a few places where you might need to update numbers of studies from the initial stages of the literature search (which were conducted by EPA).

You'll see that the two appended tables present criteria for applying RoB ratings, including both the draft OHAT guidelines and any assessment-specific guidelines. Hopefully this is helpful.

Thanks for sending the ADP; I haven't opened that file yet, but we'll take a look now.

Please let me or Audrey know if you have any questions on the attached documentation.

Dave

DAVE BURCH | Principal | 919.293.1630 office | 919.450.7372 cell | dave.burch@icfi.com | icfi.com
ICF INTERNATIONAL | 2635 Meridian Pkwy, Suite 200 | Durham, NC 27713

Bohn, Brent

From:	Cowden, John
Sent:	Thursday, August 22, 2013 1:24 PM
To:	Cowden, John; Lowit, Anna; Wolf, Doug C.; Doyle, Elizabeth; Burgess, Michele; Sams, Reeder; Ramasamy, Santhini; Dellarco, Vicki; Foster, Stiven; Lee, Janice
Cc:	Tsui-Bowen, Alethea; Cuthbertson, Becky; Mazza, Carl; Braverman, Carole; Chen, Chao; Dockins, Chris; Hodes, Colette; Axelrad, Daniel; Bussard, David; Thomas, David; Corona, Elizabeth; Burneson, Eric; Koustas, Erica; Waleko, Garland; Miller, Gregory; Pagan, Ines; Cuje, Jace; Hetrick, James; Chen, Jonathan; Bradham, Karen; Raffaele, Kathleen; Fusinski, Keith; Schumacher, Kelly; Costello, Kevin; Christensen, Krista; Servidio, Lia; Kissinger, Lon; Olsen, Marian; Maddaloni, Mark; Loughran, Michael; Shao, Nicole; Edelstein, Rebecca; Ofrane, Rebecca; Darney, Sally; Serda, Sophia; Griffin, Susan; Myers, Tom; Walsh, Debra; Strong, Jamie; Vandenberg, John; Deener, Kathleen; Olden, Kenneth; Gwinn, Maureen; Jones, Samantha; Cogliano, Vincent; Devoney, Danielle
Subject:	RE: Arsenic Coordination Committee - Quarterly Meeting - Thursday August 29th, 3pm-4pm
Categories:	Record Saved - Private

Hi everyone,

Happy Thursday! I hope that things are going well for you today. Sunny and warm down here in NC.

Our next Arsenic Coordination Committee meeting is ***next*** Thursday, August 29th from 3pm – 4pm. If you have a calendar invite for TODAY, please delete that invitation. That meeting invitation is a remnant of the "smooth" transition between Lotus Notes and Outlook. I have deleted the Lotus Notes meeting invitation on my end, but it seems to remain on folks' calendars.

At the next meeting, we would like to discuss the upcoming schedule and anticipated products for the IRIS tox review of iAs. For context, we're attaching copies of the draft planning documents submitted to the NRC. These documents are publically available from the NRC, but we've included them here to ensure everyone has copies.

The documents provide the an overview of EPA's preliminary approaches to develop the assessment (Draft iAs Assessment Development Plan) and summarize how EPA will identify and evaluate available scientific information for health effects due to exposure to iAs (Draft iAs NRC Materials). In addition, we've sent the draft report from the internal stakeholder workshop, draft report from the public meeting, and the draft planning and scoping summary.

These draft planning documents will be revised in response to NRC recommendations. The NRC guidance document on developing the iAs toxicological review is anticipated in late September.

I hope that you can make it to our next meeting. If you have any questions, feel free to contact Reeder, Janice, or me.

Have a great afternoon!

John

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Draft iAs NRC Materials.pdf

Draft iAs Assessment Dev...

iAs_Scoping_an...

iAs_Public_Stake...

iAs_Planning_an...

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Bohn, Brent

From: Cowden, John
Sent: Thursday, April 03, 2014 9:25 AM
To: Turley, Audrey
Cc: Lee, Janice; Sams, Reeder
Subject: RE: Arsenic: Discuss ICF's Proposed Approach for Meta-Analyses

Categories: Record Saved - Private

Hi Audrey,

Happy Thursday! I hope that things are going well for you today.

Would you mind adding Jeff Gift, Allen Davis, Tom Luben, and Ellen Kirrane to the invite (after accepting, I can't see the invitee list). I think Ellen, Allen, and Tom are all out of the office on Friday but it can't hurt to send them an invite.

Jeff and Allen have reviewed the materials and provided comments. I think they were be key contributors to the discussion.

Let me know if you have any questions. Have a great morning!

John

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-----Original Appointment-----
From: Turley, Audrey [mailto:Audrey.Turley@icfi.com]
Sent: Thursday, April 03, 2014 9:21 AM
To: Turley, Audrey; Mendez Jr, William; Eftim, Sorina; Cowden, John; Lee, Janice; Sams, Reeder
Subject: Arsenic: Discuss ICF's Proposed Approach for Meta-Analyses
When: Friday, April 04, 2014 2:30 PM-3:30 PM (UTC-05:00) Eastern Time (US & Canada).
Where: 1-877-423-6338 x619104#

Please let us know if you are available to discuss the proposed meta-analysis approach on Friday, April 4 at 2:30.
Audrey
<< File: Arsenic_Meta-Analyses Approach_032814.pdf >>

Bohn, Brent

From: Cowden, John
Sent: Tuesday, September 09, 2014 4:09 PM
To: Powers, Christina
Cc: Lee, Janice; Joca, Lauren
Subject: Revised lit search figure
Attachments: Gen lit search diagram - draft.pptx

Categories: Record Saved - Private

Hi Christy,

Happy Tuesday! I hope that things are going well for you today up in Ann Arbor. Take solace that no matter how bad it is to lose to ND, things are worse in Columbus!

I've put together a simplified lit search diagram for the ADP revisions, based on your version. I wanted to get your input, particularly on the MOA stuff. The goal of this figure is to be generic enough to cover both hazard ID and DR.

Let me know if you have any suggestions. Have a great afternoon!

John

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Bohn, Brent

From:	Henning, Cara <Cara.Henning@icfi.com>
Sent:	Thursday, March 14, 2013 10:57 AM
To:	Cowden, John
Cc:	Turley, Audrey; Lee, Janice
Subject:	Risk of bias questions
Attachments:	Risk of Bias Questions 031413.xlsx
Categories:	Record Saved - Private

Hi John,

Here are the Risk of Bias questions we are considering. Several questions are combined together in some categories. The table also shows the source of the questions (NTP or SAB comments). All of the SAB questions are included. The NTP questions are also represented except those that deal strictly with clinical human studies.

Hope this gives you what you need for your meeting,
Cara

CARA HENNING |
ICF INTERNATIONAL |

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Bohn, Brent

From: Fritz, Jason
Sent: Thursday, June 26, 2014 1:46 PM
To: Cowden, John; Lee, Janice
Cc: Chiu, Weihsueh
Subject: Rodent diets and development
Attachments: Odum 2001 rodent diets and development.pdf

Categories: Record Saved - Private

Heya,

Just forwarding along an article that evaluates the effects of rat diet on development…I don't get the impression that it's nearly as cut-and-dry as what I heard from today's discussion…I was surprised to hear that there was a diet which would completely invalidate a developmental study (although I am by no means a dev tox expert).

Just FYI

Thanks,
Jason

TOXICOLOGICAL SCIENCES **61**, 115–127 (2001)

Effect of Rodent Diets on the Sexual Development of the Rat

J. Odum,* H. Tinwell,* K. Jones,* J. P. Van Miller,† R. L. Joiner,‡ G. Tobin,§
H. Kawasaki,¶ R. Deghenghi,|| and J. Ashby*[,1]

**Syngenta Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, SK10 4TJ, United Kingdom; †Union Carbide Corporation, Danbury, Connecticut 06817; ‡General Electric Company, Pittsfield, Massachusetts 01201; §Harlan Teklad U.K., Bicester, Oxfordshire, United Kingdom; ¶Japanese Chemical Industries Association, Sumitomo Chemical Co., Ltd., 27–1, Shinkawa 2-chome, Chuo-ku, Tokyo 104-8260, Japan; and ||Europeptides, 9 Avenue du Marais, 95108 Argenteuil, France*

Received November 30, 2000; accepted January 23, 2001

Five rodent diets have been evaluated for their possible effect on the sexual development of the rat. Groups of 12 pregnant Alpk rats were fed one of the following combinations of diets during pregnancy and postnatally: RM3/RM1, AIN-76A/AIN-76A, RM3/AIN-76A, Teklad Global 2016 (Global)/Global and Purina 5001/Purina 5001. AIN-76A is phytoestrogen-free while the other diets contained varying amounts of phytoestrogens. The phytoestrogens genistein and daidzein were determined in the diets studied, and the concentrations found agreed with earlier estimates. RM3/RM1 was selected as the control group, as this has been used routinely in this laboratory for the past decade. Determinations were made in offspring of the times of vaginal opening and first estrus among the females, and of prepuce separation and testes descent among the males. At postnatal day (PND) 26 the females from 6 of the 12 litters were terminated and tissue weights measured. Males from 6 of the 12 litters were similarly studied at PND 68. Animals from the remaining litters were transferred to RM1 diet at PND 70. Termination of the study was at PND 128 (males) and PND 140 (females) when body weights and tissue weights were determined.

Marked differences in body weight, sexual development, and reproductive tissue weights were observed for rats maintained on AIN-76A or Purina 5001, with only minimal effects among rats maintained on the Global diet. These comparisons were against RM3/RM1 as the reference diet. However, using Purina 5001 as the reference diet reversed the direction of the differences seen when using RM3/RM1 as the reference diet. The differences observed when using RM3/RM1 as reference diet occurred mainly postnatally. In addition, the fact that similar differences were seen for the phytoestrogen-free diet, AIN-76A, and the phytoestrogen-rich diet, Purina 5001, indicate that these effects are more likely to be caused by nutritional differences between the diets that then have centrally mediated effects on rodent sexual development, rather than individual dietary components affecting peripheral estrogen receptors (ER). This proposal is supported by abolition of the uterotrophic activity of AIN-76A and Purina 5001 (relative to RM3/RM1) in the immature rat by coadministration of the gonadotrophin-releasing hormone (GnRH) antagonist Antarelix.

The present data indicate that choice of diet may influence the timing of sexual development in the rat, and consequently, that when evaluating the potential endocrine toxicity of chemicals, the components of rodent diets used should be known, and as far as is possible, controlled.

Key Words: **phytoestrogen; sexual development; rat; endocrine toxicity; rodent diet.**

The development of standardized protocols to test for hormonally active compounds is a major goal of regulatory agencies worldwide. A strategy for testing for effects on male and female sexual maturation and thyroid activity, in accordance with EDSTAC (Endocrine Disrupter Screening and Testing Advisory Committee) recommendations has recently been published (Goldman *et al.*, 2000; Stoker *et al.*, 2000). Interpretation of results from such tests requires the acquisition of background/baseline data and an understanding of factors that may cause variation of these baselines. One of these factors is the phytoestrogen content and the energy of diet administered to the rodents. Most laboratory animal diets are formulated with constituents that contain phytoestrogens (plant derived estrogenic compounds); for example, soy extract containing the isoflavones genistein and daidzein, or alfalfa, which contains coumestrol (Patisaul and Whitten, 1999). These phytoestrogens are estrogenic to rodents, causing effects such as increased uterine weight and advanced vaginal opening in immature animals, similar to effects observed with xenobiotic estrogens (Tinwell *et al.*, 2000; Bickoff *et al.*, 1962; Casanova *et al.*, 1999; Medlock *et al.*, 1995; Whitten *et al.*, 1992).

The ability of phytoestrogens to influence the outcome of endocrine toxicity evaluations is illustrated by Boettger-Tong *et al.* (1998), who reported their inability to demonstrate a uterotrophic response to estradiol in rats receiving a diet high in phytoestrogens. Thigpen *et al.* (1999) corroborated these findings and stressed the importance of dietary phytoestrogens not only in studies of uterine growth but also in evaluations of the carcinogenicity of chemicals to the rodent mammary and prostate glands. We have also reported that the rodent diet selected for use can affect control uterine weights in the immature rat uterotrophic assay (Odum *et al.*, 1997).

[1] To whom correspondence should be addressed. Fax: +44 0 1625 590249. E-mail: john.ashby@syngenta.com.

Thigpen *et al.* (1999) have suggested that the use of a semisynthetic rodent diet such as AIN-76A could eliminate the influence of phytoestrogens in laboratory-animal diets. However, when AIN-76A was fed for 3 days to immature rats, they had heavier uteri than animals maintained on our standard RM1 rat diet (Ashby *et al.*, 1999). This uterotrophic activity of AIN-76A was abolished by coadministration of the antiestrogen Faslodex, thereby confirming a direct involvement of the estrogen receptor (ER) (Ashby *et al.*, 1999). Similar uterotrophic activity was observed for Purina 5001 (Ashby *et al.*, 2001), a diet reported to contain a relatively high phytoestrogen content (Thigpen *et al.*, 1999). In addition, it was shown that the uterotrophic activity of AIN-76A could be abolished by coadministration of the gonadotrophin-releasing hormone (GnRH) antagonist Antarelix, indicating that, in addition to the involvement of ER, the uterotrophic activity is mediated centrally via effects on the hypothalamus (Ashby *et al.*, 2000). This finding for AIN-76A indicated that unknown dietary factors, in addition to phytoestrogens, can act as modulators of endocrine toxicity endpoints.

The above observations indicate that the diet selected for rodent endocrine toxicity studies may influence the outcome of those studies. Given this, and in the absence of agreement of a standard diet for use in endocrine toxicity studies, it became of interest to investigate a range of diets for their possible effects on the sexual development of male and female rats. The diets selected are commercially available and generally employed throughout the world. These were Rat and Mouse no. 3 (RM3) (used for "breeding, lactation and growth of young animals") and RM1 (a "general maintenance diet"), each being standard U.K. rodent diets, AIN-76A (a semisynthetic diet with no soy or alfalfa added), Teklad Global 2016 (a diet made from natural ingredients and containing no soy or alfalfa, intended primarily for growth and maintenance but shown in our study as supporting breeding), and Purina 5001 (a standard rodent diet used particularly in the U.S., suitable for "life-cycle nutrition" and reported to contain a high level of phytoestrogens; Thigpen *et al.*, 1999). Combinations of these diets were fed to rats throughout pregnancy and to the offspring until they reached adulthood (Fig. 1). An additional group of animals were maintained on RM3 throughout pregnancy and on AIN-76A postnatally (RM3/AIN-76A). Sentinel developmental landmarks and reproductive organ weights in the offspring were then evaluated. The diets were also tested in the immature rat uterotrophic assay in the presence and absence of the GnRH antagonist Antarelix.

MATERIALS AND METHODS

Animals. Alpk:APfSD (Wistar derived) rats, obtained from the AstraZeneca breeding unit (Alderley Park), were used in both studies. Animal studies were performed in accordance with the U.K. "Animals (Scientific Procedures) Act." Animal care and procedures were carried out according to in-house standards as described previously (Odum *et al.*, 1999).

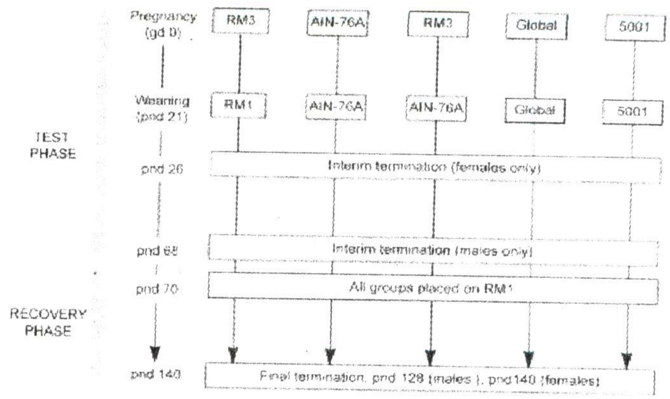

FIG. 1. Design of the sexual maturation study.

Diets and chemical. The following diets were used. Rat and Mouse No.3 (RM3), and Rat and Mouse No.1 (RM1) were obtained from Special Diet Services, Ltd., Witham, Essex, U.K. AIN-76A and Teklad Global 2016 (Global), were obtained from Harlan Teklad U.K., Bicester, Oxfordshire, U.K. Purina Chow 5001 (5001) was from Purina Mills, Inc., Richmond, IN, U.S. All diets and drinking water were available *ad libitum*. The GnRH antagonist Antarelix was a gift from Europeptides, a Division of Asta Medica, Argenteuil Cedex, France.

Dietary analysis. The diets were analyzed for genistein and daidzein content by GC-MS. Aliquots of the diets (10 pellets) were ground to a homogenous powder; 100mg of each was then extracted with 80% methanol (80 ml) by ultrasonication (3 min) followed by incubation at 60°C for 2 h and further ultrasonication (3 min). The mixtures were cooled, made up to 100 ml with methanol, and 0.1 ml samples taken and mixed with 0.05 ml methanol containing internal standards (deuterated d4-daidzein, d4-genistein, and dihydroxyflavone). Sodium acetate buffer (1 ml; 0.1 M pH 5.0) was added to the samples, which were then treated with β-glucuronidase (*Helix pomatia*, 1000 units) to a final volume of 2.5 ml and incubated overnight at 37°C. The products were then extracted with ethyl acetate (2 × 4ml) and the combined extracts evaporated to dryness. The residues were reconstituted in chloroform:heptane:methanol 10:10:1. They were then applied to short columns of Sephadex LH20, washed with chloroform:heptane:methanol 10:10:1 (4ml), and eluted with methanol. After evaporation of the methanol, the samples were derivatized for GC-MS with n-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide containing 1% t-butyldimethylsilyl chloride (0.04 ml) in acetonitrile (0.04 ml) at 65°C for 2 h. After evaporation of the solvents the residues were reconstituted in ethyl acetate (0.02 ml) for GC-MS.

GC-MS was carried out on a DB5 MS-bonded silica capillary column (10 × 0.25 mm, phase thickness 0.25 μm) using helium as carrier gas and a temperature of 70–300°C at 40°C per min. Isotope dilution MS was performed using selective ion monitoring at mass 425 for daidzein, 429 for d4-daidzein, 555 for genistein, and 559 for d4-genistein. Peak area ratios were determined for analytes and internal standards. Calibration curves were constructed and the concentrations of daidzein and genistein in the samples determined.

Sexual maturation study. The experimental design for this study is shown in Figure 1. Sixty pregnant female rats (10–12 weeks old) were assigned to 5 groups on day 0 of pregnancy (day of sperm-positive smear). Each group contained 12 pregnant females in order to achieve 10 litters per group, although this number was less than is recommended under ICH guidance criteria (where $n = 16$) and interim terminations meant that for some endpoints the numbers of litters were halved. Each group received a different diet combination through pregnancy, weaning, and up to postnatal day (PND) 68 (test phase: see Fig. 1). Birth occurred naturally and no pup culling took place before weaning on PND 21 (day of birth = day 0). At weaning, the sexes were separated and housed with littermates. All females were retained at weaning, as the female offspring from 6 of the litters in each group were killed at PND 26 (the usual endpoint of the uterotrophic assay) and sex-organ weights determined. Males were culled to 4 per litter at weaning in order to standardize to 4 animals per cage. Animals were weighed at 4-day intervals from birth until weaning, and thereafter every 7 days. Food consumption per cage was monitored throughout the study and recorded as total food consumed per cage, weekly, from which average food consumption per group was calculated.

The following developmental landmarks were monitored: eye opening (from PND 8), testis descent (TD, from PND 21), vaginal opening (VO, from PND 21) and prepuce separation (PPS, from PND 35). The age at first estrus was determined by taking vaginal smears after vaginal opening, smearing ceasing when first estrus was defined. Smearing commenced again between PND 52–69 in order to determine the percentage of days spent in estrus. When the male offspring were sexually mature (PND 68) males from 6 litters per group were killed and liver, kidney and sex organ weights determined. The remaining females were culled to 4 per litter at the same time (PND 68). On PND 70 all male and female animals were placed on RM1 diet to ascertain if any of the differences that might be seen would be reversible (Recovery Phase: Fig. 1). The males were killed at PND 128 and the females at PND 140–144, as the growth curves indicated that plateaus had been reached. Females were killed when they were in estrus (established by vaginal smears). Liver, kidney, and sex organ weights were determined for both male and female animals at termination.

Uterotrophic assay. An immature rat uterotrophic assay was carried out using weanling rats (20–21 days old on arrival) as described previously (Odum *et al.*, 1997). Animals were weaned on RM3 diet in the breeding unit and then fed RM1, AIN-76A, Global, or 5001 upon acceptance into the laboratory, and for the 4-day duration of the assay. Food consumption was monitored daily. Rats (21–22 days old at the start of dosing) received Antarelix daily for 3 days by subcutaneous injection (300 μg/kg/day) in arachis oil (AO) (dosing volume 5 ml/kg). Control animals received AO only. Animals were killed by an overdose of halothane (AstraZeneca plc) 24 h after the final dose. Uteri were removed, blotted, and weighed, as described earlier (Odum *et al.*, 1997).

Statistical methods. For the sexual maturation study, initial body weights were analyzed by variance and subsequent body weights by covariance with the initial body weight (taken at weaning). Food consumption was analyzed by variance. Organ weights were analyzed by variance and by covariance with the terminal body weights (Shirley, 1996). The proportions of animals recorded each day with developmental landmarks were analyzed by Fisher's Exact test and the observed days for the developmental landmarks were analyzed by variance. Body weights recorded at the time of observation of the landmark were also analyzed by variance. Differences from control values in all cases were assessed statistically using a 2-sided Student's t-test based on the error mean square from the analysis of variance or covariance. Analyses were carried out twice, first taking the RM3/RM1 group as control and secondly taking the 5001/5001 group as control. In all cases the litter was considered to be the statistical unit. Analyses were carried out as described in SAS (1996).

For the uterotrophic assays, uterine weights were analyzed by covariance with the terminal body weights. Terminal body weights were adjusted for covariance with initial body weights. Differences from control values (RM1 + AO) were assessed statistically using a 2-sided Student's t-test, based on the error mean square from the analysis of covariance. The individual was considered to be the statistical unit.

RESULTS

Diet Analysis

RM1, RM3, Global, and 5001 are "closed formulae" (i.e., their precise components are known only to the manufacturer) natural ingredient diets. AIN-76A is an "open formula" (i.e., its exact specification is known) synthetic ingredient diet (Knapka, 1983). The manufacturers describe RM1, RM3, Global, and 5001 as suitable for maintenance of rodents. AIN-76A was devised by the American Institute of Nutrition (AIN) in 1973 and has been widely used for many years (Reeves *et al.*, 1993). The ingredients of the five diets are shown in Table 1. With the exception of AIN-76A, all of the diets contain cereals as their primary ingredient. In contrast, AIN-76A contains, principally, sucrose and casein. The reported protein contents of RM3 and 5001 are greater than Global and RM1, as the former are described as being suitable for breeding where a higher protein content may be necessary. The calculated metabolizable energy values, which reflect the amount of energy available to the animals on consumption of the diets, are shown in Table 1. The values were similar in the natural ingredient diets (lowest, RM1; highest, Global) but that of AIN-76A was substantially higher, reflecting its high digestibility.

RM1, RM3 and 5001 contain soybean meal, which is a rich source of phytoestrogens, particularly isoflavones. Purina 5001 also contains alfalfa, which is a source of coumestrol, another phytoestrogen (Table 1). We have attempted to estimate the levels of soy and alfalfa where the manufacturer has not stated these levels. The isoflavone content of the diets (genistein and daidzein, determined by GC-MS) is shown in Figure 2. The quantities of genistein and daidzein correspond to the amounts of soybean meal in the diets: 5001 had the highest levels, followed by RM3 and RM1, while AIN-76A and Global had barely detectable levels.

Sexual Maturation Study

Data analysis. To compare effects observed with the different diets, the RM3/RM1 combination was taken as the control (reference) diet, because this combination has been used in most of our previous studies (Odum *et al.*, 1997, 1999; Tinwell *et al.*, 2000). RM3 is a diet suitable for breeding and lactation of rodents, and RM1 is more suitable for general maintenance. Statistically significant differences from the RM3/RM1 reference are described below and summarized in Table 2. Further analyses was carried out using 5001/5001 as the reference diet, because this is used in most regulatory and research studies in the U.S.

Body weights and food consumption. All animals were pregnant and all gave birth normally. There were no convincing differences in the body weights of the pregnant dams given the different diets up to birth (some intermittent differences were seen but these were not sustained). After birth, during the lactational period, dams receiving AIN-76A or Global had significantly reduced body weights (Fig. 3). Food consumption in these groups over this time period was also marginally reduced (data not shown), perhaps reflecting their higher metabolizable energy density.

Pup survival at birth was similar for all groups, but over the

TABLE 1
Ingredients, Protein Content, Metabolizable Energy, and Phytoestrogen Sources of the Diets Used in This Study

RM1	RM3	AIN-76A	Global 2016	5001
Ingredients				
Wheat Barley Wheat middlings Soybean meal Dried whey Soya oil Minerals Vitamins	Wheat Barley Wheat middlings Soybean meal Fish meal Dried whey Dried brewers yeast Soy oil Minerals Vitamins	Sucrose Casein Maize starch Maize oil Cellulose Minerals Vitamins	Wheat Maize Wheat middlings Maize gluten meal (60%) Dried brewers yeast Soy oil Limestone flour Minerals Vitamins	Maize Soybean meal Sugar beet pulp Fish meal Oats Dried brewers yeast Alfalfa Molasses Wheat germ Dried whey Meat meal Wheat middlings Animal fat Salt Limestone Minerals Vitamins
Protein content (%)				
14.7	22.3	20.0	16.7	23.4
Metabolizable energy (kJ/g)				
10.9	11.5	15.7	13.3	12.7
Phytoestrogen sources: soybean meal (S) and alfalfa (A) (% in diet)				
S: 6.0[a] A: nil[a]	S: 13.0[e] A: nil[a]	S: nil[a] A: nil[a]	S: nil[a] A: nil[a]	S: >18.0[e] A: 3.0[e]

Note. Ingredients are given in order of inclusion, i.e., first listed is present in highest amount.
[a]Accurate values.
[e]Estimated values.

postnatal period from birth to PND 21, there were slightly more litter losses in the AIN-76A group: 9/12 litters were surviving at PND 21 compared with 11/12 or 12/12 litters in the other groups. Body weights for male and female pups receiving AIN-76A were reduced at birth and up to PND 13, but the body weights had recovered to control (RM3/RM1) values by PND 21 (Figs. 4 and 5). After weaning, both groups receiving AIN-76A (AIN-76A/AIN-76A and RM3/AIN-76A) maintained significantly increased body weights compared with the control group (RM3/RM1). Male and female pups receiving Global diet had similar body weights at birth compared to the control (RM3/RM1) group, but were significantly reduced by PND 21. After weaning, by PND 36, both sexes had similar weights to the RM3/RM1 control group, and this was maintained throughout the study (Figs. 4 and 5). Male and female pups receiving 5001 diet had similar body weights to the control (RM3/RM1) group throughout the pre-weaning period, but from PND 28 onwards, body weights increased and were always heavier than the control group; however, the increase was only statistically significant up to PND 56 (Figs. 4 and 5).

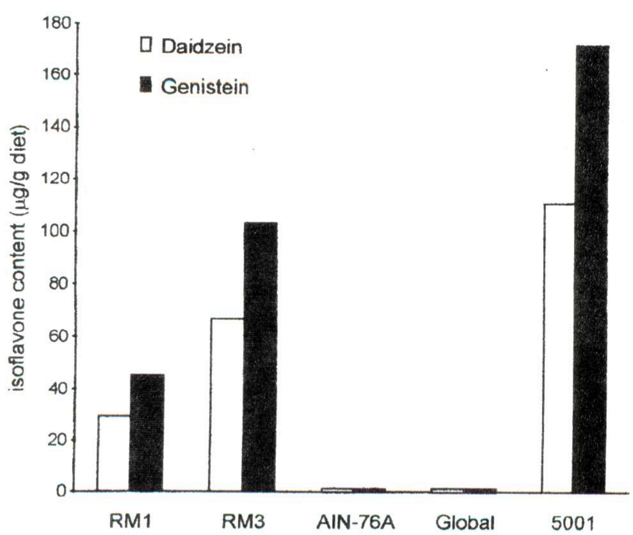

FIG. 2. Analysis of the diets for genistein and diadzein (by GC-MS, see Materials and Methods). Analyses were conducted on different batches, and on more than one occasion, with similar results. The data here represent one such set of results.

Food consumption for male and female pups in the post-weaning period is shown in Figure 6. Consumption of AIN-76A diet in the first few weeks of the study was significantly lower than control (RM3/RM1) diet animals; but by 8 weeks of age it was similar to control levels. Consumption of Global and 5001 diets was generally significantly greater than controls. At

TABLE 2
Statistically Significant Changes for Parameters When RM3/RM1 Is Taken as the Control Group

Parameter	Significance with RM3/RM1 as control			
	AIN-76A/AIN-76A	RM3/AIN-76A	Global/Global	5001/5001
Male and female developmental landmarks				
Age at TD	↑	↓	—	—
Body wt at TD	—	—	↓	—
Age at PPS	↓	↓	—	↓
Body wt at PPS	—	—	—	—
Age at VO	↓	↓	—	—
Body wt at VO	—	↓	↓	—
Day of 1st estrus	—	↓	—	↓
Female organ weights at PND 26				
Body wt at PND 26	↑	↑	↓	↑
Blotted uterus	↑	↑	↑	↑
Dry uterus	↑	↑	↑	↑
Vagina	↑	↑	↑	↑
Cervix	—	↑	—	—
Ovaries	—	↑	—	—
Male organ wts at PND 68				
Body wt at PND 68	↑	↑	—	↑
Liver	↑	↑	—	—
Kidney	↑	↑	—	↑
Testes	↓	—	—	—
Epididymides	↓	—	—	—
Seminal vesicles	—	—	—	—
Prostate	—	—	—	—
Male organ wts at PND 128				
Body wt at PND 128	↑	↑	—	—
Liver	—	—	—	—
Kidney	—	—	—	↑
Testes	↓	—	—	—
Epididymides	—	—	—	—
Seminal vesicles	—	—	—	—
Prostate	—	—	—	—
Female organ wts at PND 140				
Final body wt	↑	↑	—	—
Liver	—	—	—	—
Kidney	↑	↑	—	—
Blotted uterus	—	—	—	—
Dry uterus	↓	—	—	—
Vagina	—	—	—	—
Cervix	—	—	—	—
Ovaries	—	—	—	—

Note. TD, testis descent; PPS, preputial separation; VO, vaginal opening; wt, weight. Body weights are adjusted for covariance with initial body wts (at weaning). Organ weight data are adjusted for covariance with terminal body wts; ↓ or ↑, $p < 0.05$; —, not different.

PND 70 (Recovery Phase, during which all animals were fed RM1) feeding patterns altered. Fluctuations occurred initially in both sexes, but afterwards, all female groups started to consume similar amounts within 2 weeks, while all male groups did not achieve this equality. Males from the AIN-76A groups, who originally consumed lower quantities of diet than the RM3/RM1 control group, consumed significantly more RM1 than the control group after the diet changeover.

Developmental landmarks. The age at which all pups started and completed eye opening was determined as a general marker of development, but there were no differences between any of the diet groups. Eye opening (defined as full opening of the lid) started on PND 13 and was essentially complete by PND 17 in male and female pups from all groups (data not shown).

The age and weight at TD was not markedly altered in any of the diet groups. Inconsistent changes in the day of TD of ~1 day were seen in the AIN-76A groups and mean body weight at TD was slightly lower in the Global group (Tables 2 and 3). Significant changes in the day of PPS were seen in both

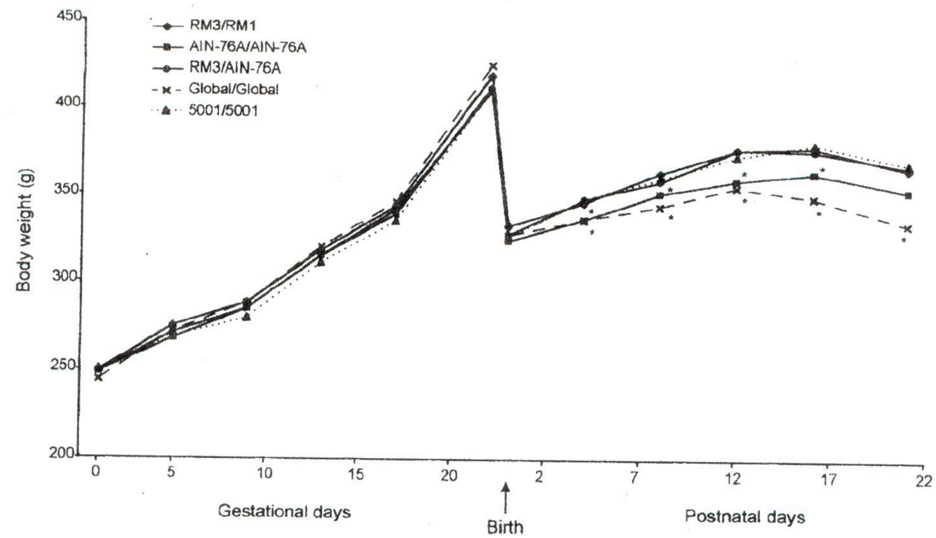

FIG. 3. Body weights of pregnant and lactating dams up to pup weaning. Gestational day (GD) 0 = the day sperm were found in the vaginal smear; birth (GD 22) = PND 0. The first diet in each line of the key was used throughout gestation and up to weaning (PND 21). The second diet in each line of the key was used after weaning, and therefore was not used in the period shown in this figure. Data are ANCOVA-adjusted means; $n = 12$ for all groups up to birth, but by PND 21, had been reduced to 9, 11, and 11 in the AIN-76A/AIN-76A, RM3/AIN-76A, and Global/Global groups, due to litter losses. *Statistically significant changes when compared with RM3/RM1 as control group, $p < 0.05$.

AIN-76A groups where PPS was advanced by ~3 days whilst body weights at PPS were similar to the RM3/RM1 controls. PPS was advanced by ~1.5 days in the 5001 group. PPS in the Global group was similar to the RM3/RM1 control (Tables 2 and 3).

The ages at onset and completion of VO followed by day of first estrus were determined. Body weights at completion of VO were also determined, although in later studies we have used body weights at onset of VO as a preferred parameter (Ashby *et al.*, 2000b). Onset of VO was accelerated by 2–3 days in both AIN-76A groups, compared to RM3/RM1, while body weight at VO was either unchanged or reduced compared to the RM3/RM1 control group. First estrus occurred earlier in the RM3/AIN-76A group (Tables 2 and 3). VO and first estrus were also advanced in the 5001 group, although the former was not statistically significant when using the litter as the experimental unit. The VO data for 5001 have previously been presented using the individual as the experimental unit, and in that case the advance in the mean day of VO was statistically significant (Ashby, 2000). Age at VO and first estrus in the Global group was unaffected (Tables 2 and 3). In all groups, a low proportion of the animals failed to complete VO, as evidenced by the retention of vaginal threads. Body weights at completion of VO, and the day of first estrus, were therefore not obtained for these animals that were excluded from all the female developmental landmark data in Table 2, but they were assessed with the other animals at termination. The total number of animals with vaginal threads was as follows: RM1/RM3, 17; AIN-76A/AIN-76A, 8; RM3/AIN-76A, 11; global, 11; and 5001, 12. The percentage of days spent in estrus during the estrus cycle was determined on all animals but was not different in any of the groups (data not shown).

Organ weights. Female pups from 6 litters from all groups were terminated at PND 26 (the usual time of termination of the immature rat uterotrophic assay), and liver, kidney and sex

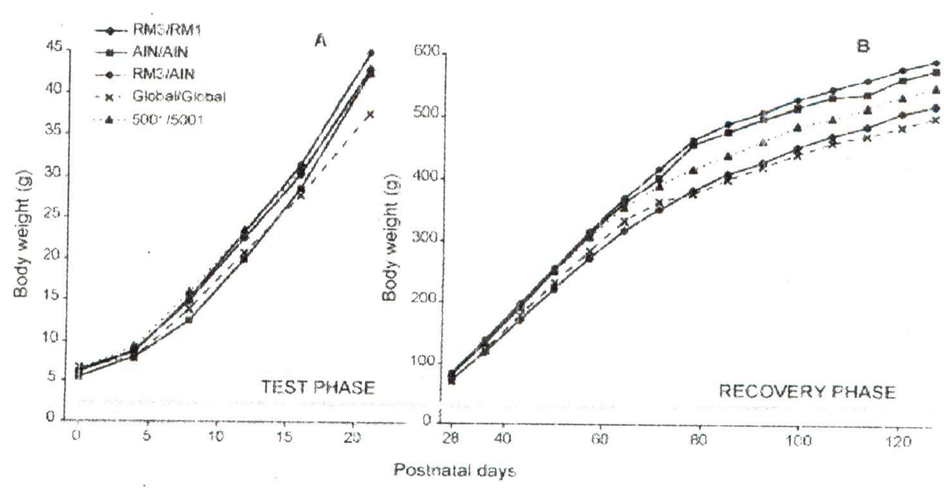

FIG. 4. (A) Body weights of male pups from birth (PND 0) to weaning (PND 21); (B) body weights of male pups from weaning to PND 126. The first diet in each line of the key was used throughout gestation and up to weaning (PND 21). The second diet in each line of the key was used after weaning. All groups were given RM1 from PND 70. Data are ANCOVA-adjusted means; $n = 12$ litters for all groups at birth but by PND 21, there were 11, 9, 11, 11, and 12 litters for RM3/RM1, AIN/AIN, RM3/AIN, Global/Global, and 5001/5001 groups, respectively. Statistical significance is not shown (for clarity) but is described in Results. The RM3/RM1 group is regarded as the control group.

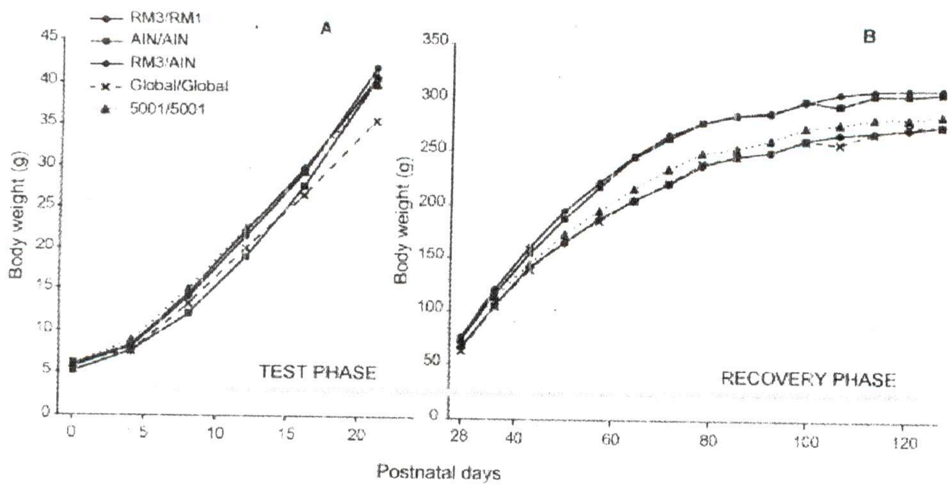

FIG. 5. (A) Body weights of female pups from birth (PND 0) to weaning (PND 21); (B) body weights of female pups from weaning to PND 126. The first diet in each line of the key was used throughout gestation and up to weaning time (PND 21); the second diet in each line was used after weaning. All groups were given RM1 from PND 70. Data are ANCOVA-adjusted means. (A): $n = 12$ litters for all groups at birth but by PND 21 there were 9, 11, and 11 litters for AIN-76A/AIN-76A, RM3/AIN-76A, and Global/Global groups, respectively; (B): $n = 6$ litters for all groups except AIN-76A/AIN-76A where $n = 5$. Statistical significance is not shown (for clarity) but is described in Results. The RM3/RM1 group is regarded as the control group.

organ weights were determined. Adjusted body weights, uterine and vaginal weights differed significantly for all the diets compared to the reference RM3/RM1 group. Adjusted cervix weight was also increased for the RM3/AIN-76A group, as was the adjusted ovarian weight for the RM3/AIN-76A group (Tables 2 and 4).

Male pups from 6 litters from each group were terminated at PND 68. Liver, kidney, and male sex organ weights were determined. The most marked differences were seen for the AIN-76A/AIN-76A group and no changes were seen for the global group (Tables 2 and 5). The adjusted weights of the testes and epididymides were decreased in the AIN-76A/AIN-76A group.

At PND 70 (recovery phase) all groups were placed on RM1 to determine whether the body and organ weight changes observed at PND 68 would be maintained or reversed. The remaining males were terminated at PND ~128, after ~8 weeks after the diet change to RM1. Adjusted body weights for AIN-76A/AIN-76A and RM3/AIN-76A were still significantly increased relative to the control RM3/RM1 group. Adjusted testes weights were still significantly decreased in the AIN-76A/AIN-76A group and adjusted kidney weights remained significantly elevated in the 5001/5001 group (Tables 2 and 6).

The remaining females were terminated at PND ~140, 10 weeks after the diet change to RM1. Adjusted body weights for AIN-76A/AIN-76A and RM3/AIN-76A remained elevated relative to the control RM3/RM1 group. Adjusted kidney weights were increased in these two groups, but there were no other notable changes in any of the other groups compared to the RM3/RM1 control group (Tables 2 and 7).

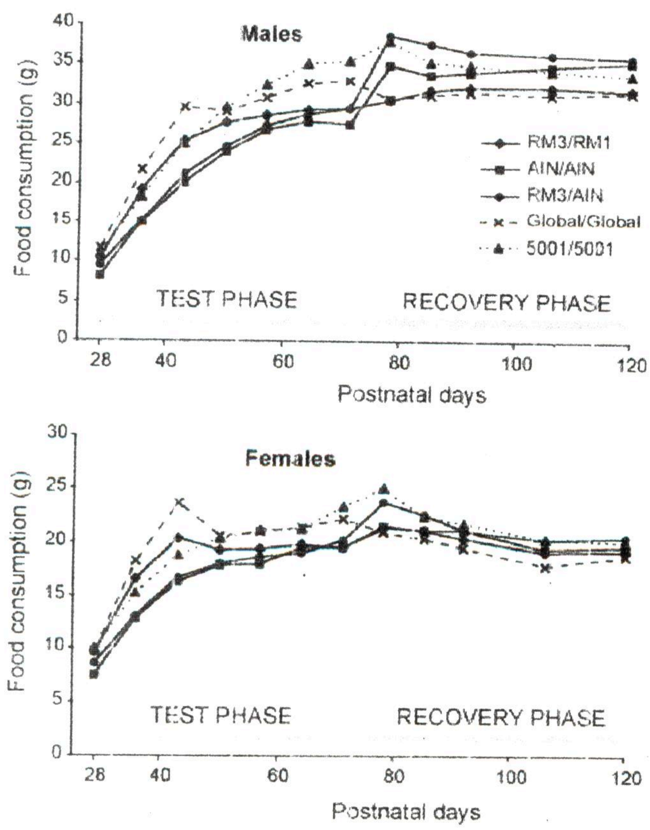

FIG. 6. Food consumption (post-weaning) for male and female pups. The first diet in each line of the key was used throughout gestation and up to weaning; the second diet in each line was used after weaning. All groups were given RM1 from PND 70. Data are means, n as shown in Figures 4 and 5. Statistical significance is not shown (for clarity) but is described in Results. The RM3/RM1 group is regarded as the control group.

Uterotrophic Assay

Rats receiving AIN-76A or 5001 had significantly heavier terminal body weights than those receiving RM1 or Global. Food consumption for diets other than RM1 was increased (by 24–40%) relative to the RM1 + AO (vehicle only) control group. Absolute and adjusted uterine weights were significantly increased in animals receiving AIN-76A or 5001 and vehicle only compared to the RM1 + AO control group. After

TABLE 3
Developmental Landmark Results for Male and Female Pups

	RM3/RM1 (control)	AIN-76A/AIN-76A	RM3/AIN-76A	Global/Global	5001/5001
Male developmental landmarks					
Age at testes descent (days)	23.6 ± 1.0	24.4 ± 1.3[a],*	22.8 ± 0.9*	24.4 ± 0.8	23.3 ± 0.8
Body wt at testes descent (g)	51.4 ± 3.3	54.8 ± 4.9	52.5 ± 4.7	47.5 ± 4.7*	54.4 ± 5.6
Age at PPS (days)	45.5 ± 1.7	43.0 ± 1.9*	42.5 ± 0.9*	45.1 ± 1.4	43.9 ± 1.6*
Body wt at PPS (days)	199.3 ± 11.5	194.1 ± 13.4	204.2 ± 9.5	193.9 ± 14.9	207.5 ± 8.8
Total no. pups	43	30	41	38	44
No. litters	11	9	11	11	12
Female developmental landmarks					
Age at onset of VO (days)	34.9 ± 1.5	32.3 ± 0.7*	31.3 ± 0.5*	34.5 ± 1.8	33.8 ± 0.8
Body wt at completion of VO (g)	111.2 ± 10.5	104.2 ± 6.8	97.4 ± 5.3*	99.0 ± 6.9*	110.8 ± 5.8
Day of 1st estrus	39.2 ± 2.6	37.5 ± 2.4	34.7 ± 2.2*	38.2 ± 2.0	36.1 ± 1.3*
No. pups	17	15	18	24	19
No. litters	6	5	6	6	6

Note. Data are mean ± SD (n = number of litters).
[a]Two rats had undescended testes; testes descent data exclude these.
*$p < 0.05$. Statistically significant changes compared with RM3/RM1 as control group.

Antarelix treatment no uterotrophic effects were seen for any of the diets, uterine weights generally being lower than the RM1 + AO control group (Table 8).

DISCUSSION

Uncertainties regarding the optimum diet for use in rodent endocrine disruption studies, and whether the phytoestrogen contents of diets should influence this decision (Ashby *et al.*, 1999; Thigpen *et al.*, 1999), led to the present study.

Soy proteins (as used in some rodent diets) contain various levels of estrogenic phytoestrogens, of which the major are generally found to be genistein and daidzein (Thigpen *et al.*, 1999). Of the diets studied here, Purina 5001 was found to have the highest genistein and daidzein content: approximately 180 μg and 150 μg/g diet, respectively. These values are somewhat lower than those reported by Thigpen *et al.* (1999; 214 μg and 277 μg/g diet, respectively), but are similar to those reported by Casanova *et al.* (2000) for NIH-07 diet (160 μg and 144 μg/g diet, respectively). Variation in reported levels may result

TABLE 4
Organ Weights for Females Terminated at PND 26

Parameter		RM3/RM1 (control)	AIN-76A/AIN-76A	RM3/AIN-76A	Global/Global	5001/5001
Body wt at PND 26 (g)	abs[a]	58.1 ± 2.9	58.6 ± 5.2	64.3 ± 5.3	50.5 ± 10.4	65.5 ± 4.7
	adj[b]	56.2	62.0*	62.2*	53.4*	64.1*
Blotted uterus (mg)	abs[c]	21.8 ± 3.86	30.4 ± 3.8	43.2 ± 5.6	24.9 ± 3.8	42.4 ± 4.2
	adj[d]	22.5	30.8*	41.2*	28.8*	39.9*
Dry uterus (mg)	abs[c]	4.6 ± 0.5	6.0 ± 0.7	8.4 ± 0.9	5.2 ± 0.6	8.1 ± 0.8
	adj[d]	4.6	6.0*	8.2*	5.7*	7.8*
Vagina (mg)	abs[c]	25.2 ± 4.0	30.0 ± 2.3	38.9 ± 3.5	25.8 ± 5.6	35.6 ± 3.2
	adj[d]	25.9	30.4*	36.7*	30.1*	32.8*
Cervix (mg)	abs[c]	6.7 ± 2.53	7.7 ± 0.4	10.1 ± 1.3	5.9 ± 0.6	9.2 ± 1.3
	adj[d]	6.6	7.8	9.6*	6.8	8.6
Ovaries (mg)	abs[c]	31.4 ± 2.4	33.4 ± 2.9	36.4 ± 2.4	29.2 ± 2.0	35.6 ± 3.8
	adj[d]	31.7	33.6	35.2*	31.4	34.2
No. pups		31	21	28	37	31
No. litters		6	4	5	5	6

[a]Absolute body weights (mean ± SD, n = number of litters).
[b]Body weights adjusted for covariance with initial body wts (at weaning).
[c]Absolute organ weights (mean ± SD, n = number of litters).
[d]Organ weights adjusted for covariance with terminal body weights.
*Statistical significance (adjusted data only) was tested using RM3/RM1 as control group; $p < 0.05$. Absolute data was not tested.

TABLE 5
Organ Weights for Males Terminated at PND 68

Parameter		RM3/RM1 (control)	AIN-76A/AIN-76A	RM3/AIN-76A	Global/Global	5001/5001
Body wt at PND 68 (g)	abs[a]	362.6 ± 23.7	394.9 ± 20.7	407.2 ± 10.1	369.5 ± 27.7	404.5 ± 12.2
	adj[b]	355.4	411.3*	387.7*	372.2	403.1*
Liver (g)	abs[c]	17.5 ± 0.9	21.2 ± 1.5	21.5 ± 1.3	17.3 ± 1.2	19.8 ± 1.0
	adj[d]	18.7	20.9*	20.6*	18.2	19.1
Kidney (g)	abs[c]	2.5 ± 0.1	3.0 ± 0.2	3.6 ± 0.1	2.6 ± 0.3	3.5 ± 0.3
	adj[d]	2.7	2.9*	3.4*	2.7	3.3*
Testes (g)	abs[c]	3.28 ± 0.10	3.00 ± 0.14[e]	3.40 ± 0.14	3.14 ± 0.15	3.28 ± 0.14
	adj[d]	3.34	2.99[e,]*	3.36	3.18	3.24
Epididymides (mg)	abs[c]	801 ± 41	754 ± 19[e]	817 ± 30	791 ± 50	827 ± 32
	adj[d]	821	749[e,]*	802	805	814
Seminal vesicles (g)	abs[c]	1.07 ± 0.13	1.10 ± 0.10	1.29 ± 0.09	1.16 ± 0.09	1.29 ± 0.09
	adj[d]	1.16	1.08	1.23	1.22	1.24
Prostate (mg)	abs[c]	311 ± 26	347 ± 36	381 ± 38	300 ± 43	327 ± 27
	adj[d]	336	341	363	318	311
Total no. pups		19	16	14	16	19
No. litters		5	5	5	5	6

[a]Absolute body weights (mean ± SD, n = number of litters).
[b]Body wts adjusted for covariance with initial body weights (at weaning).
[c]Absolute organ wts (mean ± SD, n = number of litters).
[d]Organ wts adjusted for covariance with terminal body weights.
[e]Data for 2 rats with undescended testes are excluded from testis and epididymis weights.
*Statistical significance (adjusted data only) was tested using RM3/RM1 as control group; $p < 0.05$. Absolute data was not tested.

from differences in analytical method, of which there are several for genistein and daidzein, and the variation in genistein and daidzein that is found in different batches of soybean meal. Boettger-Tong *et al.* (1998) reported levels of 210 μg and 140 μg/g genistein and daidzein, respectively, for a particular and nonrepresentative batch (rogue batch) of their standard diet, which they described as "a non-purified diet from a major U.S. manufacturer." According to Boettger-Tong

TABLE 6
Organ Weights for Males Terminated at PND 128

Parameter		RM3/RM1 (control)	AIN-76A /AIN-76A	RM3/AIN-76A	Global/Global	5001/5001
Body wt at PND 128 (g)	abs[a]	508.8 ± 30.5	558.1 ± 37.5	588.5 ± 41.7	500.4 ± 43.6	536.2 ± 29.5
	adj[b]	520.9	574.8*	592.8*	502.7	549.7
Liver (g)	abs[c]	18.1 ± 1.8	19.7 ± 2.4	20.2 ± 2.0	17.1 ± 1.8	18.4 ± 1.1
	adj[d]	19.2	18.8	18.1	18.6	18.5
Kidney (g)	abs[c]	2.8 ± 0.3	3.2 ± 0.2	3.4 ± 0.2	2.8 ± 0.3	3.3 ± 0.2
	adj[d]	3.00	3.05	3.17	2.99	3.28*
Testes (g)	abs[c]	3.55 ± 0.279	3.44 ± 0.20	3.63 ± 0.37	3.58 ± 0.26	3.53 ± 0.19
	adj[d]	3.67	3.34*	3.40	3.75	3.53
Epididymides (mg)	abs[c]	1274 ± 57	1241 ± 25	1315 ± 48	1300 ± 85	1280 ± 73
	adj[d]	1290	1229	1286	1321	1280
Seminal vesicles (g)	abs[c]	1.56 ± 0.27	1.51 ± 0.16	1.59 ± 0.18	1.58 ± 0.15	1.70 ± 0.16
	adj[d]	1.62	1.47	1.48	1.66	1.7
Prostate (mg)	abs[c]	512 ± 43	498 ± 87	507 ± 81	465 ± 65	469 ± 75
	adj[d]	515	495	500	469	469
Total no. pups		24	15	24	24	23
No. litters		6	4	6	6	6

[a]Absolute body weights (mean ± SD, n = number of litters).
[b]Body weights adjusted for covariance with initial body weights (at weaning).
[c]Absolute organ weights (mean ± SD, n = number of litters).
[d]Organ weights adjusted for covariance with terminal body wts.
*Statistical significance (adjusted data only) was tested using RM3/RM1 as control group; $p < 0.05$. Absolute data was not tested.

TABLE 7
Organ Weights for Females Terminated at PND 140

Parameter		RM3/RM1 (control)	AIN-76A/AIN-76A	RM3/AIN-76A	Global/Global	5001/5001
Body wt at PND 140 (g)	abs[a]	281.8 ± 10.7	302.0 ± 13.0	318.9 ± 5.2	275.0 ± 13.4	287.4 ± 14.9
	adj[b]	279.8	305.4*	317.2*	277.4	287.1
Liver (g)	abs[c]	9.0 ± 0.6	9.0 ± 0.4	9.9 ± 6	8.6 ± 0.6	9.0 ± 0.6
	adj[d]	9.2	8.7	9.3	9.0	9.2
Kidney (g)	abs[c]	1.7 ± 0.1	2.2 ± 0.1	2.3 ± 0.2	1.7 ± 0.1	1.9 ± 0.1
	adj[d]	1.8	2.2*	2.2*	1.8	1.9
Blotted uterus (mg)	abs[c]	456.3 ± 17.8	435.8 ± 79.7	471.7 ± 74.3	413.1 ± 46.9	478.1 ± 41.2
	adj[d]	475.2	418.5	424.3	444.2	487
Dry uterus (mg)	abs[c]	88.1 ± 26.9	81.7 ± 10.2	91.5 ± 10.7	80.6 ± 4.8	90.9 ± 6.5
	adj[d]	90.6	79.4*	85.2	84.7	92.1
Vagina (mg)	abs[c]	152.0 ± 9.1	167.2 ± 10.9	153.7 ± 18.7	162.1 ± 13.8	155.9 ± 16.0
	adj[d]	154.1	165.3	148.0	165.5	156.9
Cervix (mg)	abs[c]	76.4 ± 11.0	75.1 ± 19.3	78.8 ± 19.4	75.7 ± 8.2	86.8 ± 4.5
	adj[d]	78.0	73.6	74.7	78.3	87.6
Ovaries (mg)	abs[c]	113.7 ± 9.9	115.2 ± 5.0	116.4 ± 8.9	113.0 ± 9.7	106.7 ± 9.5
	adj[d]	115.2	113.8	112.6	115.5	107.4
Total no. pups		24	14	23	24	24
No. litters		6	4	6	6	6

[a]Absolute body weights (mean ±SD, n= number of litters).
[b]Body weights adjusted for covariance with initial body weights (at weaning).
[c]Absolute organ weights (mean ±SD, n= number of litters).
[d]Organ weights adjusted for covariance with terminal body weights.
*Statistical significance (adjusted data only) was tested using RM3/RM1 as control group; $p < 0.05$. Absolute data was not tested.

et al. (1998), the phytoestrogens present in their rogue batch of diet led to significant increases in control immature rat uterine weights leading to a dramatic loss of assay sensitivity. Purina 5001, NIH-07, and Boettger-Tong's rogue batch of diet also contained alfalfa, which is a potential source of the phytoestrogen coumestrol (Bickoff *et al.*, 1962, Tinwell *et al.*, 2000), but

TABLE 8
Uterotrophic Assay for Weanling Rats Administered Different Diets in the Presence or Absence of Antarelix

Treatment		Terminal body wt (g)		Blotted uterus wt (mg)	*n*
RM1 + AO (control)	abs[a]	50.0 ± 2.5	abs[c]	20.5 ± 2.7	12
	adj[b]	50.4	adj[d]	21.6	
AIN-76A + arachis oil	abs[a]	56.4 ± 3.9	abs[c]	33.0 ± 7.3	12
	adj[b]	56.3*	adj[d]	32.4*	
Global + arachis oil	abs[a]	51.0 ± 3.9	abs[c]	21.8 ± 3.1	12
	adj[b]	50.8	adj[d]	22.6	
5001 + arachis oil	abs[a]	58.2 ± 4.8	abs[c]	36.7 ± 5.9	12
	adj[b]	57.7*	adj[d]	35.6*	
RM1 + antarelix	abs[a]	50.9 ± 3.1	abs[c]	15.1 ± 1.2	12
	adj[b]	51.0	adj[d]	16.0	
AIN-76A + antarelix	abs[a]	57.6 ± 2.7	abs[c]	16.7 ± 1.5	12
	adj[b]	57.4	adj[d]	15.7	
Global + antarelix	abs[a]	50.4 ± 3.3	abs[c]	16.2 ± 1.0	12
	adj[b]	50.9	adj[d]	17.1	
5001 + antarelix	abs[a]	57.9 ± 4.0	abs[c]	19.3 ± 4.5	12
	adj[b]	58.1	adj[d]	18.3	

[a]Absolute body weights (mean ± SD, n = number of rats).
[b]Body weights adjusted for covariance with initial body weights (at the start of the study).
[c]Absolute uterus weights (mean ± SD, n =number of rats).
[d]Organ weights adjusted for covariance with terminal body weights.
*Statistical significance (adjusted data only) for *increases* over control values was tested using RM1 + arachis oil as control group; $p < 0.05$. Absolute data was not tested.

this was not analytically determined in any of the above evaluations, or in the present study.

All of the diets studied here produced changes in one or another of the developmental landmarks or reproductive tissue weights, relative to the RM3/RM1 control animals, these changes being generally most marked for Purina 5001 (phytoestrogen-rich) and AIN-76A (phytoestrogen-free), and least marked for the Global diet. These changes are shown in Table 2 and are not discussed in detail here, the main point of relevance to emerge being that the choice of rodent diet can affect the sexual development in rats in a way that is not related directly to the phytoestrogen contents of the diets. As an example, the uterotrophic effects elicited by Purina 5001 and AIN-76A (Table 2) are of a similar magnitude to the uterotrophic effects of weak synthetic estrogens such as nonylphenol in animals maintained on our standard RM/RM1 diet (Odum *et al.*, 1997). It is of interest that the majority of the effects induced by the AIN-76A/AIN-76A combination were also observed for the RM3/AIN-76A combination, suggesting that the postnatal period was the most sensitive to dietary influences on sexual development. Pup survival in the immediate postnatal period was also reduced with AIN-76A, indicating that this diet is not very suitable for breeding and lactation. The uterotrophic activity of AIN-76A (Table 8) was reported earlier (Ashby *et al.*, 1999, 2001) and is of particular interest given that this formulation contains nondetectable levels of phytoestrogens. Although the effects reported here for AIN-76A in female rats are typical of estrogenic compounds (Ashby *et al.*, 1997; Goldman *et al.*, 2000), the advance in PPS seen for both it and Purina 5001 in male rats was unexpected, given that estrogens generally delay PPS (Ashby and Lefevre, 2000; Stoker *et al.*, 2000). This difference argues for a different (and perhaps nonestrogenic) mechanism of action for this effect.

In related studies, we have recently found that soy-based infant formulae also produced uterine growth and advanced VO and first estrus in immature female rats (Ashby *et al.*, 2000). The levels of phytoestrogens present in the soy formulae were insufficient to account for these activities, and uterine growth did not occur in ovariectomized rats given soy formulae (Ashby *et al.*, 2000). However, the estrogen antagonist Faslodex inhibited these effects of the soy formulae, indicating that they were associated with increased exposure to estrogen. Use of a GnRH antagonist abolished these effects of the infant formula, indicating a centrally mediated mechanism of action associated with increased hypothalamic excretion of GnRH leading to premature synthesis of endogenous estrogen in immature rats, itself leading to premature entry of the rats into puberty (Ashby *et al.*, 2000). A similar mechanism may explain the effects reported here for these several diets. Thus, the uterotrophic effects of AIN-76A and Purina 5001 were abolished by the GnRH antagonist Antarelix, and body weights of the pups receiving AIN-76A or Purina 5001 were consistently heavier than those receiving RM3/RM1 or the Global diets. Further, both male and female sexual development occurred earlier in the AIN-76A and Purina 5001 groups. The reduced testes and epididymides weights in the AIN-76A/AIN-76A group cannot, however, be explained in these terms. Transfer of all of the test groups to RM1 diet (recovery phase) led to general maintenance of the body weight changes recorded for the two AIN-76A groups, the final body weights of the Global and Purina 5001 groups being similar to those of the RM3/RM1 control group.

The dietary component(s) responsible for effects reported here have yet to be established. There may be no single causative factor, because the sexual development of rats fed AIN-76A or Purina 5001 was similar, despite the composition of the diets being markedly different (Table 1). In particular, although centrally mediated increases in pup growth rates are suggested here to be a potentially critical stimulus to advanced sexual development, the metabolizable energies of the different diets, and the amount of diet consumed, do not provide an obvious explanation for the effects reported. Glucose and sucrose have been eliminated as the uterotrophic species in infant formulae and the AIN-76A diet (Ashby *et al.*, 2000).

Purina 5001 is widely used in the U.S. for both regulatory and research studies, and we therefore reanalyzed the present database using Purina 5001 as the reference diet (Table 9). This reversed the direction of the effects for the other diets, including RM3/RM1. This illustrates that the choice of reference diet is as important as the choice of a special diet for individual studies.

In our studies with infant formulae, we observed a correlation between the quantity of formula consumed by immature rats and mice and the magnitude of the resultant uterotrophic effect (Ashby *et al.*, 2000). That finding suggests that the amount of an endocrine-active diet (e.g., AIN-76A and Purina 5001) consumed by rodents may influence the timing of puberty. In some toxicity studies, palatability problems lead to the test animals consuming less diet than do the matched controls. Further, a general requirement for an adequate toxicity study is that the test animals should weigh less at the end of the study than do the matched control animals. Both of these situations may affect the timing of puberty of the test animals, with the chance of weak endocrine toxicity effects being inappropriately concluded for the test agent. In such situations it would be valuable to have access to food-intake data for the test and control animals, but this is often not available. In order to evaluate this speculative source of uncertainty, it would be necessary to conduct a rodent sexual maturation study that included a food-restricted group of animals and a group with enhanced food intake (via the use of a flavoring agent). Such a study, which will also monitor the potential involvement of insulin and leptin and changes in body fat, is currently being planned.

In conclusion, administration of different diets to rats can affect the timing of both male and female sexual development. Phytoestrogens are not necessarily or wholly responsible for these effects. Although the present data indicate that choice of

TABLE 9

Statistically Significant Changes for Parameters, When 5001/5001 Is Taken As the Control Group

Parameter	Significance with 5001/5001 as control			
	AIN-76A/AIN-76A	RM3/AIN-76A	Global/Global	RM3/RM1
Male and female developmental landmarks				
Age at TD	↑	—	↑	—
Body wt at TD	—	—	↓	—
Age at PPS	—	↓	—	↑
Body wt at PPS	↓	—	↓	—
Age at VO	—	↓	—	—
Body wt at VO	—	↓	↓	—
Day of 1st estrus	—	—	—	↑
Female organ weights at PND 26				
Body wt at PND 26	—	—	↓	↓
Blotted uterus	↓	—	↓	↓
Dry uterus	↓	—	↓	↓
Vagina	—	↑	—	↓
Cervix	—	—	—	—
Ovaries	—	—	—	—
Male organ weights at PND 68				
Body wt at PND 68	—	—	↓	↓
Liver	↑	↑	—	—
Kidney	↓	—	↓	↓
Testes	↓	—	—	—
Epididymides	↓	—	—	—
Seminal vesicles	↓	—	—	—
Prostate	—	↑	—	—
Male organ weights at PND 128				
Body wt at PND 128	—	—	—	—
Liver	—	—	—	—
Kidney	↓	—	↓	↓
Testes	—	—	—	—
Epididymides	—	—	—	—
Seminal vesicles	—	—	—	—
Prostate	—	—	—	—
Female organ weights at PND 140				
Final body wt	↑	↑	—	—
Liver	—	—	—	—
Kidney	↑	↑	—	—
Blotted uterus	—	—	—	—
Dry uterus	↓	—	—	—
Vagina	—	—	—	—
Cervix	—	—	—	—
Ovaries	—	—	—	—

Note. TD, testis descent; PPS, preputial separation; VO, vaginal opening. Body weights are adjusted for covariance with initial body weights (at weaning). Organ weight data are adjusted for covariance with terminal body weights; ↓ or ↑, $p < 0.05$; —, not different.

diet may influence some of the markers of endocrine toxicity, it cannot at this stage be concluded that any of the diets studied are inappropriate for use in endocrine toxicity studies. However, the components of rodent diets should be known, and as far as is possible, controlled. For investigators who do not have an historical database to prejudice by a change in diet, it would seem prudent to select a diet with low phytoestrogen levels. Among those suggested here to be suitable are cereal-based soy-free or low-soy diets, such as Teklad Global 2016, or the cereal-based soy-free version of NIH-07 used by Casanova *et al.* (2000), or low-soy diets, such as RM1. These diets give low control uterine weights in weanling rats, and this reduces the chance of these diets influencing the outcome of endocrine toxicity studies. However, when selecting a diet containing even small amounts of soybean meal, consideration should be given to the possibility that the use by a manufacturer of soybean meals of varying phytoestrogen content could produce resulting experimental variation.

ACKNOWLEDGMENTS

We thank S. Kirk for the statistical analyses and Dr. M. Morton (Bioclinical Services, Ltd, Cardiff, U.K.) for the isoflavone analysis. We are grateful to the

Alkyl Phenol Ethoxylate (APE) Research Council, the Endocrine Working Group of the Japanese Chemical Industries Association, and the Food Standards Agency (U.K.) for financial contributions to the conduct of this study.

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Bohn, Brent

From: Sams, Reeder
Sent: Wednesday, January 23, 2013 10:47 AM
To: Walsh, Debra
Cc: Flowers, Lynn; Vandenberg, John; Cogliano, Vincent; Chiu, Wuehsueh; Lee, Janice; Cowden, John
Subject: Slides for NAS

Categories: Record Saved - Private

Debra,

Attached are the draft slides Janice, John, and I prepared for the iAs NAS meeting on Thursday, January 24th. Any specific or overall recommended changes you may have would be great. We will be doing a practice presentation this afternoon and will likely have some revisions afterwards. Also, we are incorporating some revisions suggested by John and Weihsueh.

Best Regards,
Reeder

Jan NAS presentations Sa...

Reeder L. Sams II, Ph.D.
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Bohn, Brent

From: Turley, Audrey <Audrey.Turley@icfi.com>
Sent: Friday, August 23, 2013 5:31 PM
To: Cowden, John; Lee, Janice
Subject: SQ Summary: Dermal
Attachments: SQReport_Arsenic_Dermal_Summary.docx; ATT00001.htm; SQReport_Arsenic_Dermal_Detailed.docx; ATT00002.htm

Follow Up Flag: Follow up
Flag Status: Completed

Categories: Record Saved - Private

Hi guys - dermal study quality attached. Thanks! Audrey

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Bohn, Brent

From: Henning, Cara <Cara.Henning@icfi.com>
Sent: Tuesday, June 18, 2013 2:15 PM
To: Cowden, John; Lee, Janice
Cc: Turley, Audrey; Eftim, Sorina; Blain, Robyn; Henning, Cara
Subject: SQ tables with include/exclude draft decisions
Attachments: BladderEffects_SQ Tables_with_include_exclude.docx; EndocrineEffects_SQ Tables_with_include_exclude.docx; NervousEffects_SQ Tables_with_include_exclude.docx

Categories: Record Saved - Private

Hi John,

Here are the study quality tables for endocrine, neuro, and bladder effects with the "include/exclude" logic applied to them. Again, let us know if you want to talk through it.

Thanks,
Cara

CARA HENNING |
ICF INTERNATIONAL ;

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Bohn, Brent

From: Cowden, John
Sent: Monday, May 19, 2014 11:12 AM
To: Turley, Audrey
Cc: Lee, Janice; Sams, Reeder; Gift, Jeff; Burch, Dave
Subject: Status of Hazard Id meta-analysis materials

Categories: Record Saved - Private

Hi Audrey,

Happy Monday! I hope that things are going well for you today.

Were the draft hazard ID meta-analysis materials sent on Friday (05/16/14)? We haven't received any materials - I even checked the infamous "Junk" folder - so wanted to make sure things weren't bouncing back.

We're planning to send the draft dose-response and hazard ID meta-analysis materials up for workgroup review today at noon.

Let me know if you have any questions. Have a great morning!

John

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Bohn, Brent

From: Turley, Audrey <Audrey.Turley@icfi.com>
Sent: Monday, July 22, 2013 2:39 PM
To: Cowden, John; Lee, Janice
Cc: Henning, Cara; Blain, Robyn; Eftim, Sorina
Subject: Study Quality - Endocrine
Attachments: sq_endocrine_full_20130722.docx; sq_endocrine_summary_20130722.docx

Follow Up Flag: Follow up
Flag Status: Flagged

Categories: Record Saved - Private

Hi John and Janice,
Here are the final study quality files for the endocrine studies (summary table and full rationales).
Audrey

AUDREY TURLEY |
ICF INTERNATIONAL |

Bohn, Brent

From: Cowden, John
Sent: Thursday, January 02, 2014 10:11 AM
To: Turley, Audrey; Mendez Jr, William; Blain, Robyn
Cc: Lee, Janice; Sams, Reeder; Powers, Christina
Subject: FW: Draft iAs MOA list
Attachments: 2013 12 09 As MOA Summaries.docx

Categories: Record Saved - Private

Hi everyone,

Happy Thursday! Here is the DRAFT DRAFT DRAFT table Christy has pulled together for MOA stuff.

Have a great afternoon!

John

John Cowden, Ph.D.
Hazardous Pollutant Assessment Group (HPAG)
National Center for Environmental Assessment (NCEA)
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From: Cowden, John
Sent: Wednesday, December 18, 2013 11:34 AM
To: Hetes, Bob
Cc: Sams, Reeder; Powers, Christina
Subject: FW: Draft iAs MOA list

Hi Bob,

Happy Wednesday! I hope that things are going well for you today.

Thanks for taking the initiative on the AOPs! If we can tap into the CSS resources that would be great. Here's our initial steps to develop qualitative summaries for the various MOAs for iAs. We're filling in the slots with manuscripts and starting a lit search, but this chart might be helpful as you move forward.

Let me know if you have any questions. See you this afternoon!

John

John Cowden, Ph.D.
Hazardous Pollutant Assessment Group (HPAG)
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From: Powers, Christina
Sent: Monday, December 09, 2013 9:30 AM
To: Cowden, John

Cc: Powers, Christina
Subject: Draft iAs MOA list

Hi John,

Happy Monday! Hope you had a great weekend and your week is off to a good start.

Attached is a very rough draft of the MOA list for iAs that we discussed. This was developed primarily from the NRC Interim Report but I believe that it encompasses the potential MOAs discussed in the previous draft assessments given my brief reviews of those documents.

I've included a few notes in the document that hopefully clarify the information, but please don't hesitate to contact me with any questions . I'm also happy to revise this draft if it's useful before we move forward with developing an example of the qualitative MOA text we discussed for the document.

Thanks,
Christy

Christy Powers, PhD

Postdoctoral Fellow

National Center for Environmental Assessment (B 220-I)

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Bohn, Brent

From: Gift, Jeff
Sent: Friday, November 15, 2013 10:55 AM
To: Cowden, John
Cc: El-Masri, Hisham; Lee, Janice; Davis, Allen; Shao, Kan; bruce_allen@frontier.com; Uhl, Mike; Nair, Ravi; Brown, Michael E.; Jarabek, Annie; Sams, Reeder; Hetes, Bob; Burgoon, Lyle
Subject: FW: Evidence tables for iAs
Attachments: As_EvidenceTables_Short_10312013.docx

Categories: Record Saved - Private

Thanks John! This will be helpful to get a general feel for the type of data we are looking at assessing. Mostly human and mostly relative risk numbers I'm guessing. I'm forwarding the tables to Bruce and the others that will be at the Monday meeting. I'll also attach them to the calendar invite.

Jeff Gift, Ph.D.
National Center for Environmental Assessment
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919-541-4828
919-541-0245 (fax)
gift.jeff@epa.gov

From: Cowden, John
Sent: Friday, November 15, 2013 10:37 AM
To: Gift, Jeff
Cc: Lee, Janice; Sams, Reeder; Shao, Kan
Subject: Evidence tables for iAs

Hey Jeff,
Happy Friday! I hope that things are going well for you today.
Here are the draft epi evidence tables for iAs. All of these data exist in DRAGON database, so we should be able to export to any format you need. Let me know if you have any questions.
Have a great morning!
John

John Cowden, Ph.D.
Hazardous Pollutant Assessment Group (HPAG)
National Center for Environmental Assessment (NCEA)
U.S. Environmental Protection Agency - RTP
(919) 541-3667

Bohn, Brent

From: Cowden, John
Sent: Wednesday, March 27, 2013 8:45 AM
To: Henning, Cara
Cc: Turley, Audrey; Fedak, Kristen; Blain, Robyn; Lee, Janice; Sams, Reeder
Subject: RE: "overall" risk of bias

Categories: Record Saved - Private

Hi Cara,

Happy Wednesday! I hope that things are going well for you today. But emails at 11:30PM??? Do you guys ever sleep? ☺

Absolutely yes, include the criteria. We're definitely interested in seeing the thought process. Insights from these first attempts at study quality evaluation would be helpful, even if we don't end up using an overall score.

Let me know if you have any questions. Have a great morning!

John

John Cowden, Ph.D.
Hazardous Pollutant Assessment Group (HPAG)
National Center for Environmental Assessment (NCEA)
U.S. Environmental Protection Agency - RTP
(919) 541-3667

From: Henning, Cara [mailto:Cara.Henning@icfi.com]
Sent: Tuesday, March 26, 2013 11:23 PM
To: Cowden, John
Cc: Turley, Audrey; Fedak, Kristen; Blain, Robyn
Subject: "overall" risk of bias

Hi John,

I know we have discussed that we will not present the overall risk of bias conclusion for each study in the report we are delivering to you. However, do you want to include how we arrived at the overall score in the section describing the study quality evaluation methodology? That is, we wouldn't give the answers, but we would tell the consistent criteria we used to get to the anwers?

Thanks,
Cara

CARA HENNING|
ICF INTERNATIONAL |

Bohn, Brent

From: Turley, Audrey <Audrey.Turley@icfi.com>
Sent: Friday, September 20, 2013 2:07 PM
To: Lee, Janice; Cowden, John; Blain, Robyn; Eftim, Sorina
Subject: RE: Arsenic "other" studies
Attachments: Arsenic_Unique Studies in Other Category.xlsx

Follow Up Flag: Follow up
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Hi all –
As Robyn noticed, the file I sent on Monday was NOT the one I intended to send. Please use this one to review the studies in the "Other" category and we can discuss what to do with them next week.
Audrey

From: Turley, Audrey
Sent: Monday, September 16, 2013 5:19 PM
To: lee.janices@Epa.gov; cowden.john@Epa.gov
Subject: Arsenic "other" studies

Hi guys,
Here are the studies currently in the "other" category that haven't yet been evaluated. Several of them are just miscategorized (there are some liver effects, for example), but the others are clinical chemistry.
Audrey

AUDREY TURLEY |
ICF INTERNATIONAL |

Bohn, Brent

From:	Cowden, John
Sent:	Tuesday, May 28, 2013 11:30 AM
To:	Turley, Audrey; Lee, Janice; Sams, Reeder
Cc:	Henning, Cara
Subject:	RE: Arsenic Study Quality for Bladder Cancer
Categories:	Record Saved - Private

Thanks Audrey!

John Cowden, Ph.D.
Hazardous Pollutant Assessment Group (HPAG)
National Center for Environmental Assessment (NCEA)
U.S. Environmental Protection Agency - RTP
(919) 541-3667

From: Turley, Audrey [mailto:Audrey.Turley@icfi.com]
Sent: Tuesday, May 28, 2013 11:20 AM
To: Cowden, John; Lee, Janice; Sams, Reeder
Cc: Henning, Cara
Subject: Arsenic Study Quality for Bladder Cancer

John,
We'll be able to send you the study quality tables for bladder cancer by the end of the day today.
Audrey

AUDREY TURLEY |
ICF INTERNATIONAL |

Bohn, Brent

From:	Henning, Cara <Cara.Henning@icfi.com>
Sent:	Wednesday, May 29, 2013 9:17 AM
To:	Cowden, John; Turley, Audrey
Cc:	Henning, Cara
Subject:	RE: Arsenic Study Quality for Bladder Effects
Categories:	Record Saved - Private

Hi John,

For crying out loud, as we like to say in the south. I removed my hyperlinks...that's the best hypothesis I've heard, and I would be shocked if anyone clicked on those anyway. The fun part about our emails going to your junk folder is that we never know when it will happen...

Thanks,
Cara

CARA HENNING |
ICF INTERNATIONAL |

From: Cowden, John [mailto:Cowden.John@epa.gov]
Sent: Wednesday, May 29, 2013 8:56 AM
To: Henning, Cara; Turley, Audrey; Lee, Janice; Sams, Reeder
Subject: RE: Arsenic Study Quality for Bladder Effects

Hi Audrey and Cara,

Happy Wednesday! I hope that things are going well for you today.

Thanks for materials, even though they got sorted to the junk file! ☺ Janice and I think that the hyperlinks in your email signatures are to blame, but it's just a hypothesis. We'll look over the evidence tables and plan to meet later this week.

Have a great morning!

John

John Cowden, Ph.D.
Hazardous Pollutant Assessment Group (HPAG)
National Center for Environmental Assessment (NCEA)
U.S. Environmental Protection Agency - RTP
(919) 541-3667

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Bohn, Brent

From: Turley, Audrey <Audrey.Turley@icfi.com>
Sent: Wednesday, May 29, 2013 3:03 PM
To: Lee, Janice; Cowden, John; Sams, Reeder
Cc: Henning, Cara
Subject: RE: Arsenic Study Quality for Bladder Studies - Path Forward

Categories: Record Saved - Private

Does Tuesday work for you guys? We're free from 1 until 4:30.
Audrey
-----Original Appointment-----
From: Lee, Janice [mailto:Lee.JaniceS@epa.gov]
Sent: Wednesday, May 29, 2013 2:45 PM
To: Turley, Audrey
Subject: Declined: Arsenic Study Quality for Bladder Studies - Path Forward
When: Monday, June 03, 2013 10:00 AM-11:00 AM (UTC-05:00) Eastern Time (US & Canada).
Where: At EPA